Characterization and utilization of young coconut waste (*Cocos nucifera* L) for manufacturing fermented plant extracts having potential as natural fertilizer and pesticide

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Abstract
Fermented Plant Extracts (FPE) had been produced by the utilization of young coconut waste (Cocos nucifera L) activated by Effective Microorganism-4 (EM-4). The FPE has been used as natural fertilizer and pesticide. A chemical profiling of young coconut waste was determined by phytochemical screening and GC-MS for organic content, X-Ray Fluorescence (XRF) and Atomic Absorption Spectrometry (AAS), spectrophotometry UV-Vis for C content and Kjeldahl method for N content. A variation of EM-4 volume was applied to optimize the producing of FPE.

The result indicated that the young coconut waste contained organic compound as alkaloid and tannins and anorganic content as N, P, K that formed FPE. The hight content of N, P, K, C in FPE was found in the mixture of 200 g young coconut waste, 45 mL EM-4, 25 mL red sugar 1 Kg/L and 980 mL water for 15 days. The FPE was applied to Ipomoea reptana plant, it could increase of plant height, a number of leaves and N, P, K in soil and a durability against insect attack compared to without FPE. This study demonstrates that the FPE of young coconut waste has a potential as natural fertilizer and pesticide.

Keywords: Young Coconut Waste, Organic Fertilizer, EM-4, Natural Pesticide.

Introduction

Fertilizer which is widely used at this time is inorganic fertilizer because nutrients from inorganic materials are more easily absorbed and have a high content. However, the continuous use of high-dose inorganic fertilizers over time has negatively impacted the soil and the environment[1,2]. Therefore one way to overcome the negative impact of inorganic fertilizer use is the use of organic fertilizer.

Liquid organic fertilizer is a fertilizer derived from the remaining animals or plants and humans who have undergone fermentation or decay. This fermentation process utilizes the EM-4 bio-activator to produce liquid fertilizer. The EM-4 solution has an enormous number of microorganisms, about 80 bacterias that can increase the number of soil microbes producing bio-activators (hormones and enzymes) improving soil conditions and quality and accelerating the composting process[3-6]. One of the plants that can be utilized to be used as liquid fertilizer with the help of additional activator is coconut.

Fermented Plant Extract (FPE) is the result of fermentation of plants by utilizing bio-activator EM-4 and brown sugar as an additional source of microbes that function to accelerate the degradation of samples to be fermented so that the extract is produced. In this fermentation process, the complex organic elements will be broken down into simpler compounds such as sugar, glycerol, fatty acids and amino acids. With the content, the FPE can be one of alternative as natural fertilizer and bio-pesticide which are environmentally friendly because it suppresses pathogen attack to increase the availability of nutrients for plants and antioxidants[7].

Plants are the most common source in the manufacture of bio-pesticides, which produce secondary metabolite compounds. This bio-pesticide was obtained by using fermentation technique. This fermentation process can increase total phenolics, total flavonoids and antibacterial activity[8]. The advantages of using bio-pesticide are: (1). Non-hazardous and safe for the environment (2). Effective in small and large quantities (3). Can contribute when used in integrated pest management (IPM) program.

Coconut is a plant grown in lot in the tropics area such as Indonesia. Coconut productivity in 2015 according to data of agricultural information center amounted to 15.20 billion grains/year. The high productivity of coconut is proportional to the waste produced. Research on the utilization of coconut waste as a liquid organic fertilizer has been done[9]. The purpose of this study is to find the utilities of young coconut waste to produce fermented plant extracts (FPE) by using EM-4 as an activator that can be used as natural fertilizer and pesticides.

Material and Methods

Collection and Preparation Sample: Young coconut waste was collected from Lubuk Minturun area, Padang, Indonesia. The young coconut waste was cleaned and divided into 3 parts (shell, husk and mixed type waste). All parts were ground to the size of 1x1 cm. A gravimetric method was used to determine the water content of each part.

Ingredients: Selenium mixture, sulfuric acid (H_2SO_4) Merck, sodium hydroxide (NaOH) Merck, boric acid (H_3BO_3) Merck, Conway indicator, chloride acid (HCl 37%) Merck, glucose Merck, potassium dichromate ($K_2Cr_2O_7$) Merck, potassium hydrogen phosphate (KH_2PO_4) Merck, ascorbic acid ($C_6H_8O_6$) Merck, ammonium heptamolybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}.4H_2O$) Merck, potassium antimonyl tartrate $K(SbO)C_4H_4O_6.1/2H_2O$ Merck and Whatmann-41 filter paper.

Equipments: The equipments in this reseach were anaerob fermentation equipments (plastic bottle size 2 L as digesters, plastic pipe size 1 cm diameter gas distributor composter and small plastic bottle size 600 mL), analytical balance (KERN and Sohn), pH meter (Metrohm pH Lab), tools of Kjeldahl method (destruction, distillation and titration), Atomic Absorption Spectrophotometer (Agilent model 240 AA), XRF (XRF epsilon 3, Germany), Spectrophotometer UV-Vis (Genesys 20) and Chromatographic Analysis using GC-MS (GCMS- QP2010 Plus) Chromatographic separation was performed in capillary column by RTX 5 MS with dimensions of column 30 cm x 0.25 mm x 0.25 µm, Carrier gas: Helium (purity>99.999%) Carrier gas flow rate: 1mL/min, Injector temperature: 320^{0}C.

Characterization of Young Coconut Waste: Characterization of young coconut waste with phytochemical, XRF and GC-MS was done to know the content of sample. The secondary metabolites as flavonoid, phenolic, steroid, terpenoids, alkaloids and tannins were identified by the phytochemical screening and GC-MS. The macronutrient content of the sample was measured by by XRF.

Producing Fermented Plant Extract (FPE)

Variant of Young Coconut Waste: The fermented plant extract (FPE) was made from 200 g each of sample (shell, husk and the mixed of shell and the husk) from young coconut waste and add 25 mL of EM-4 as an activator, 25 mL brown sugar solution 1 kg/L and 1000 mL of water. The mixture was shaken frequently and incubated with anaerobic condition in the dark. The gas produced during the fermentation process was streamed through a tube into a water container.

Variant of EM-4 Volume: The purpose of this fermentation process is to obtain the optimum volume of EM-4 as bio-activator which produces high levels of N, P, K, C-total. The fermentation was done by varying the volume of EM-4. The composition to produce the FPE was shown in table 1.

Determination of N, P, K, C-total of Fermented Plant Extracts (FPE): The macronutrient was investigated using the method of the Kjeldahl method for nitrogen, Walkley and Black method[10] for carbon content (C-total), molybdenum blue methods[11] for phosporus whereas the determination of levels of potassium was carried out using AAS (Atomic Absorption Spectrophotometer).

Application of Fermented Plant Extract (FPE) to *Ipomoea reptana* Plant: The purpose of this study is to know the effect of FPE on the growth of *Ipomoea reptana*. The seed of 15 days old of *Ipomoea reptana* was transferred to polybags containing soil. The FPE solution (50 mL, 5 mL/L, *V/V*) was sprayed to *Ipomoea reptana* for 30 days. The arrangement of planting of *Ipomoea reptana* was shown in figure 1.

Table 1
Composition of component to produce FPE

S.N.	Weight of Young Coconut Waste (g)	Water (mL)	Red Sugar solution1kg/L (mL)	EM-4 (mL)
1.	200	1010	25	15
2.	200	995	25	30
3.	200	980	25	45
4.	200	965	25	60

Note:

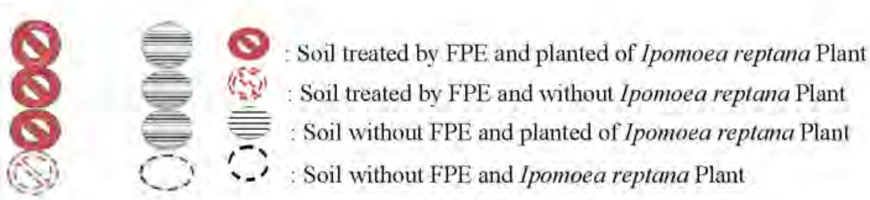


Figure 1: Design of *Ipomoea reptana* Plant Polybags

Observation of the effect of FPE on *Ipomoea reptana* growth: The observed object in this study was the height of bark and the leaf number of *Ipomoea reptana* plant and a durability against the insect attack on the 10 days after plant (DAP), 20 DAP and 30 DAP.

Analysis of Soil Content: The measurement of macronutrient content and pH in the soil were determined by the same method with FPE.

Results and Discussion

Results of Characterization Young Coconut Waste Phytochemical Test of Young Coconut Waste: Phytochemical tests were performed on young coconut waste to identify the presence of secondary metabolites from samples as a reference to know whether young coconut waste samples could potentially be as natural pesticides or not. Phytochemical test results can be seen in table 2.

Table 2
Secondary Metabolite Compound in Young Coconut Waste

S.N.	Secondary Metabolite Compounds	Test Result
1.	Alkaloids	+
2.	Steroids	-
3.	Terpenoid	-
4.	Flavonoids	-
5.	Tannins	+
6.	Phenolic	-

The alkaloids and tannins are positively contained in young coconut waste while terpenoids, steroids and flavonoids are declared negatively present in this sample. Phytochemical test results on table 2 suggested that the presence of secondary metabolite compounds in young coconut waste can make young coconut waste potentially a natural pesticide. Alkaloids are the largest secondary metabolites found in high-level plants. The alkaloid compounds can protect plants from an attack of parasites or plant predators. Some alkaloids can be toxic to other organisms as well as potentially as antimicrobial[12].

Tannins are called an effective compound as antibacterial and also have a strong potential to inhibit the growth of pathogenic bacteria[13]. In addition, tannins also serve as an anti-pest for plants to prevent insects and fungi and can function as a biological antioxidant[14]. Tannins have an antibacterial activity associated with its ability to inactivate the microbial cell adhesion, inactivate enzymes and interfere with transport of proteins in the inner lining of the cells. Tannins also have a target on the polypeptide cell wall of pest pathogens so that the formation of cell walls becomes less perfect. This causes the bacterial cells to become lysed by osmotic and physical pressure, so the bacterial cells will die[15]. Based on the results of phytochemical tests one can say that young coconut waste has the potential as a bio-pesticide.

Element and Oxide Content inYoung Coconut Waste: Characterization of young coconut waste samples by using XRF aimed to look at the nutrient content of the sample used in the manufacturing of PFE. This analysis was performed on samples.

Based on table 3, the sample of young coconut waste contains both macro and micro nutrients with high potassium content of 20.81 %. This is in accordance with XRF test results that have been done by previous researchers, where the element of potassium is the element with the highest level of 1. 21%[23]. So, it can be said that young coconut waste has potential as organic fertilizer because coconut contains many chemical elements such as nitrogen, carbon, potassium, calcium, magnesium and other micro nutrients.

Fermentation is the process of breaking up organic compounds into simple compounds involving microorganisms. Fermentation is any kind of metabolic process (enzyme, oxidized microorganism, reduction, hydrolysis, or other chemical reactions) making chemical changes to an organic substrate by producing the final products. The principle of this fermentation is the organic waste material, which is young coconut waste. There are two types of bacteria involved such as facultative bacteria that convert the substrate into glucose during the initial decomposition process and the obligate bacteria that respond in the final decomposition process. It can affect the N, P, K and C values of the resulting PFE.

Results of Secondary Metabolite in Fermented Plant Extractionfrom Young Coconut Waste: The sample analysis using GC-MS aims to determine the content of secondary metabolite compounds and organic acids present in FPE. The results of analysis of the secondary metabolite content and organic acid compounds found in FPE are shown in figure 2.

The results of analysis using GC-MS are shown in table 4. The FPE contains secondary metabolite compounds such as phenolic, alkaloids, aldehydes and carboxylic acids. Phenolic is one of the compounds having many benefits for living things. Based on phytochemical characterization, young coconut waste contained alkaloids and tannins. Alkaloid has antimicrobial activity by inhibiting esterase, DNA, RNA polymerase and cell respiration playing a role in DNA intercalation[16].

While as an antifungal, biologically alkaloids cause cell membrane damage. Phenolic is an organic compound known to actively inhibit some types of bacteria and fungal[24]. Phenolics can inhibit bacterial growth by inhibiting protease enzyme activity, inhibiting enzymes envelope transport proteins in the bacterial cell and destruct or inactivate the functions of material genetic[15].

In addition to the presence of phenolic and alkaloid content in FPE, it also contains organic acids such as hexanedioic acid and benzaldehyde. The organic substances contained in this FPE can control the plant disturbing organism. The compound contained in FPE has potential as a natural pesticide.

Effect of Variant Young Coconut Waste on Macro Nutrient and pH in FPE: Organic liquid fertilizers are formed by the fermentation process that degrade the organics contained in the sample by EM-4 microorganisms. The quality of this liquid fertilizer is not only influenced by microorganisms but also by the raw materials, bio-activators and also techniques. The length of the fermentation process will have an impact on the quality of produced liquid fertilizer[17]. The result of macronutrient of liquid fertilizer on the material variation can be seen in table 5.

Table 3
Element and Oxide Content in Young Coconut Waste

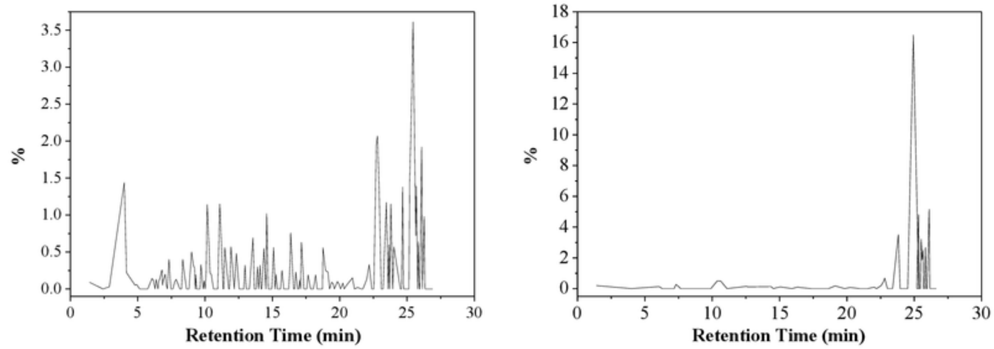
S.N.	Element	%	Oxide	%
1.	P	10.34	P_2O_5	16.24
2.	K	29.60	K_2O	20.81
3.	Mg	6. 92	MgO	9.12
4.	S	5.59	SO_3	9.14
5.	Al	4.82	Al_2O_3	6.89

Table 4
The result of Analysis of Secondary Metabolic Compound with GC-MS.

S.N.	Compounds	Coconut waste Before Fermentation		FPE	
		%	Rt	%	Rt
1.	Phenol,2-methoxy-3-methyl(CAS)-Cresol,2-methoxy-3-methylphenol-1H-indol	0.13	6.6	-	-
2.	Phenol, 4-methoxy-(CAS)	0.08	4.8	-	-
3.	Phenol-3,4-dimethoxy-(CAS)3,4-Dimethoxyphenol	0.22	7.3	-	-
4.	2-Propanol,1-Phenoxy-3-phosphino	0.34	13.5	-	-
5.	Glyceryl Tridocasahexaenote	0.12	7.0	-	-
6.	Pyrrolidine	0.14	1.4	0.33	1.41
7.	9-Octadecenamide, (Z)-(CAS)oleoamide	0.58	18.7	-	-
8.	Guanidine, dodecyl-,monoacetate	0.22	18.9	-	-
9.	1,2-Benzenedicarboxylic acid, bis (2-methoxyethyl) Ester	-	-	0.19	15.03
10.	1,2-Benzenedicarboxylic acid, diethyl ester	-	-	0.13	10.82
11.	1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (CAS) Bis (2-ethylhexyl) phthalate	-	-	0.04	6.14
12.	Hexanedioic acid, bis (2-ethylhexyl) ester (CAS) Bis (2-ethylhexyl) adipate	-	-	0.44	19.12
13.	Benzaldehyde, 2,5-bis[(trimethylsilyl)oxy](CAS)2,5-Dihydroxybenzaldehyde	-	-	0.04	6.03
14.	Trimetylsilyl Ester of 5-methyl-2-trimethylsylyloxy-benzoic Acid	-	-	3.46	25.51

Table 5
The Effect of Variant Young Coconut Waste on Macro Nutrient and pH

S.N.	Variants of young Coconut Waste	N-Total %	P-Total%	K-Total%	C-Organic %	pH
1.	Husk	0.391	0.026	1.297	2.394	4.53
2.	Shell	0.458	0.031	2.235	1.910	4.12
3.	Husk and Shell	0.547	0.032	2.031	2.291	4.41



a b

Fig. 2: Chromatograms GC–MS of a. Coconut Waste before Fermentation and b. FPE

Table 5 shows the results of analysis of FPE which proves that FPE compositions contains the macronutrient where the generated FPE can be used as liquid organic fertilizer because it can increase the amount of soil microbes producing bioactive substances that can improve the condition and quality of the soil which will turn an effect to the plant growth[3,4]. The values of N, P, K and C-Organic on the material variation showed that the macronutrient content of FPE did not indicate a considerable difference. From the data, the highest N and P values are owned by the mixture (husk + shell) of 0.547 % N and 0.032 % P. This is the basis for the selection of mixed materials as the base material on the FPE manufacture with EM-4 variations.

In this study, pH of each material was measured after the fermentation process ended. It can be seen from the data that the pH of each material used was not too different, ranging from pH 4. This pH is classified as acid due to the influence of organic acids produced by the decomposition process of organic materials in the fermentation process. pH of the produced liquid fertilizer ranged from 3.3- 4.0.

This FPE was fermented with the help of an EM-4 activator. This process takes place anaerobically. During the fermentation process, the gas is formed which is characterized by solidifying the digester and the gas is removed from the digester through a plastic pipe that has been connected to the bottle containing the residual gas reservoir of the fermented product. During the fermentation process, both physics and chemistry occurred. Physical changes that occur are the change of solution color and the resulting odor. This fermentation process usually occurs for 15-45 days[17].

FPE formed through a fermentation process will also increase the carbon content (C), nitrogen (N), potassium (K_2O), phosphate (P_2O_5). The presence of secondary metabolite compounds such as indole acetic acid (auxin) produced during fermentation by fungi is an important growth hormone for plants[19]. This is the reaction of the nutrient formation during the fermentation process:

$$\text{Protein} + E. \xrightarrow{\textit{Proteinase}} \text{TP} + \text{NADP} + NH_3 + \text{Energy}$$

$$NH_3 + 3O_2 \xrightarrow{\textit{Nitrosomonas}} 2HNO_2 + 2H_2O + \text{Energy}$$

The reaction of NO_3-forming elements absorbed by the plant is:

$$2HNO_2 + O_2 \xrightarrow{\textit{Nitrobacter}} 2HNO_3 + 2H_2O + \text{energy}$$

While to obtain phosphate, the phosphate solvent bacterial (*Pseudomonas, sp)* utilizing ATP is formed at the beginning of the fermentation process:

$$\text{ATP} + \text{glucose} \xrightarrow{\textit{Pseudomonas sp}} \text{ADP} + \text{Glucose 6-phosphate}$$

$$\text{Glucose 6-phosphate} + H_2O \rightleftharpoons \text{glucose} + \text{phosphate}$$

Table 6 shows the values of N, P and K optimum obtained at 45 mL volume of EM-4 in a row of 0.858 %, 0.0324 % and 2.832 % respectively. While the addition of EM-4 with volume 60 mL tends to decrease the value of N, P, K and C-organic equal to 0.816 %, 0.027 %, 2.222 % and 2.015 %. It is shown that the higher volumes of bio-activators used do not always produce high N, P, K and C-Organic[9]. It is also influenced by the performance of microorganisms present in EM4 with available energy sources on the using wastes and molasses where the microorganism reaches its optimum conditions at a volume of 45 mL EM-4.

The pH value decreased with the addition of EM-4, it means the resulting liquid fertilizer solution is getting acidic because of the high EM4 volume. It is influenced by the acidic bio-activator EM-4 and the activity of a number of microorganisms involved in the fermentation process to convert organic matter into organic acids[20]. A bacterial activity of nitrification converts ammonia to nitrate which causes increasing of nitrogen element in fermentation as well as microbial activity such as *Lactobacillus sp.* and *streptomyces sp.*, mushroom decomposers of cellulose and yeast which can re-model phosphorus, thus affecting the nutrient content of produced liquid fertilizer.[21]

Effect of FPE on *Ipomoea reptana* Plant

Effect of FPE on *Ipomoea reptana* Plant Height: Fermented Plant Extracts from Young Coconut Wastewere were applied to the *Ipomoea reptana* plant and watering are time a day. This watering serves to see the effect of FPE on the growth of *Ipomoea reptana* plants with observation parameters of plant height. Observations were made on 10 days after planting (DAP), 20 DAP and 30 DAP and then compared.

Figure 3 shows that giving of young coconut waste FPE can increase the growth of plants height compared to without FPE. This happens because of the amount of soluble nutrient content in FPE solution. ETT application in Chinese cabbage plants and marigold flowers showing an increase in growth shown by the increase of plant height[25]. In addition, the application of organic fertilizer to soybean (Glycine max, L) showed an increase in height and number of leaves[21].

Effect of FPE on Number of *Ipomoea reptana* Leaves: Nitrogen nutrients are needed for plants for the formation of plant vegetative parts such as roots and leaves. Nitrogen is needed in relatively large quantities by plants. Based on the results of analysis of the average number of the leaf of plants in *Ipomoea reptana*, the application of FPE increased the number of leaves of *Ipomoea reptana* plants compared without the FPE. The results of treatment on *Ipomoea reptana* plants can be seen in figure 4.

Table 6
Effect of EM-4 Volume on Macro Nutrient Contents and pH in FPE

S.N.	EM-4 Volume (mL)	N-Total %	P-Total%	K-Total %	C-Organic %	pH
1.	15	0.481	0.030	2.466	2.192	5.12
2.	30	0.586	0.030	2.597	2.156	4.60
3.	45	0.858	0.032	2.832	2.090	4.35
4.	60	0.816	0.027	2.222	2.015	4.00

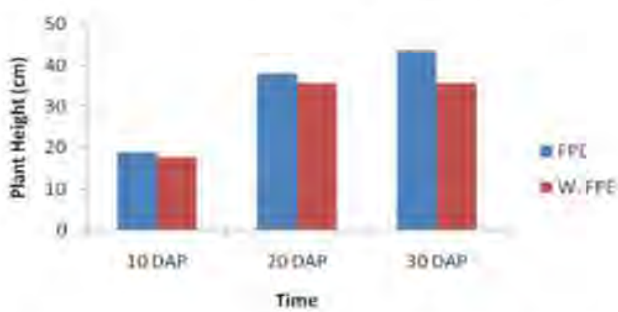


Figure 3: Effect of FPE on *Ipomoea reptana* Plant Height
Note : FPE : Plant with Fermented Plant Extract
W. FPE : Plant without Fermented Plant Extract

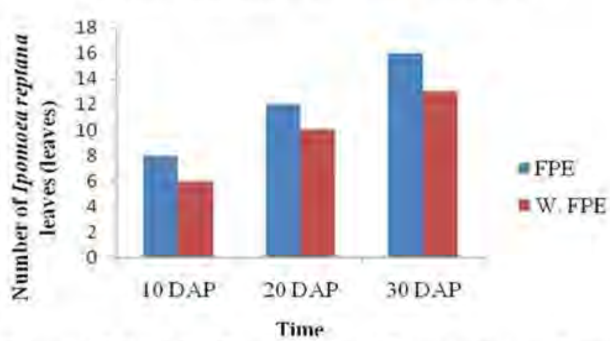


Figure 4: Effect of FPE on Number of *Ipomoea reptana* Leaves

Observations were made every 10 DAP, 20 DAP and 30 DAP. Nitrogen can increase growth and can provide a good effect for plants. Leaves in plants require relatively high concentrations of nitrogen for vegetative growth such as leaf growth, a leaf cell, root and leaf number. The utilization of organic fertilizer in soybean plants (Glycine max, L) gives a significant impact on the growth of plant height, the number of leaves and stem diameter because the content of organic fertilizer can stimulate the growth and availability of elements that can give a direct impact on the plant[21].

Effect of FPE on *Ipomoea reptana* Plant Disease: This observation component of the intensity of the disease attack was observed on the symptoms of the illness that appeared i.e. yellowing, the leaves rolled up and the leaves contracted. Based on observations for 30 days, the incidence of the disease is most prevalent in *Ipomoea reptana* plants without treated by FPE but *Ipomoea reptana* plants treated with FPE was not attacked by disease as seen in figure 5.

This is because the resulting FPE contains secondary metabolite compounds such as tannins and alkaloids, tannins are one of the phenolic compounds. Tannins and alkaloid are used as antibacterial and antifungal on the plant which can inhibit bacterial growth by inhibiting protease enzyme activity, inhibiting enzymes envelope transport proteins in the bacterial cell and destruct or inactivate the functions of material genetic[15-24].

Effect of FPE on Soil Macronutrient N, P, K C-org and pH: Macronutrient N,P,K,C (%) and pH in the soil before and after treated by FPE were listed in table 7.

Table 7 shows that FPE can increase soil nutrient N, P, K and C-organic content because the microorganisms contained in FPE can improve the physical, chemical and biological properties of the soil by increasing the microbes in the soil and activating enzymes[3,4]. Increasing levels of macro elements in the soil after application of FPE are also due to the addition of the bio-activator used as EM-4 and the degradation of the sample during the fermentation process with bio-activator microorganisms. It has also been proved[21] that the use of bio-activators in the manufacture of organic fertilizers can improve soil conditions, especially the levels of P and soil pH which will affect plant growth.

pH is one of the factors influencing the activity of microorganisms because it affects the availability of minerals needed by plants. Based on table 7, there was a decrease in pH 0.3 before and after FPE application, this is due to the addition of FPE to the soil in which the resultant FPE with EM-4 volume is optimum 60 mL acidic with a pH level of 4. Low levels of pH of the resulting FPE all due to the process of volatilization and decomposition of microorganisms into organic acids and the release of ammonia due to mineralization of organic matter. Other processes such as immobilization produce ammonium and conversion of ammonium into nitrates also result in increased acid content material[21].

Conclusion

Based on the results, it can be concluded that young coconut waste contained metabolite compounds, macronutrient having potential to produce FPE by EM-4. FPE from young coconut waste has pH 4.35, contains macronutrient (N, P, K, C-Organic) and organic acid that can be used as a natural fertilizer and pesticide.

Figure 5: Effect of FPE on *Ipomoea reptana* Plant Disease a. *Ipomoea reptana Plant* without FPE and b. *Ipomoea reptana Plant* treated with FPE

Table 7
Macronutrient N, P, K, C (%) and pH of soil before and after treated by FPE

S.N.	Soil	% N	% P	% K	% C	pH
1.	Before Treated with FPE	0.947	2.102	0.147	16.301	6.5
2.	After Treated with FPE	1.286	2.764	0.174	20.910	6.2

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